Drinking Water Early Warning Detection and Monitoring Technology Evaluation and Demonstration

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ABSTRACT

Drinking water sources have in recent years come under increasing scrutiny, with issues ranging from ecological impacts to public health and national security. In response to these concerns and facilitated by advanced electronics and fast computers, biomonitors are being developed that can assess the toxicity of water samples by monitoring living organism behavior. The U.S. EPA is currently in the process of conducting research on various biosensors at the U.S. EPA Test and Evaluation (T&E) Facility in Cincinnati, Ohio.

The purpose of this research is to evaluate and demonstrate the ability to reliably monitor source water quality using living biological organisms as sensors. Biological organisms such as *Daphnia magna (D. Magna)* change behavior dramatically from calm movement typically observed in non-polluted water to hyper-activity in water with certain pollutants. It is known that different organisms vary in their sensitivity to different substances. Other organisms, such as clams or algae, exhibit changes to various pollutants. The U.S. EPA is currently evaluating several sensors including a Daphnia Toximeter, Algae Toximeter, Clam Monitor and other Fish Monitors. These sensors measure subtle responses of these organisms and use the measured information to calculate a "toxicity index." The instrument can be set to provide "alert" and "alarm" status at predetermined toxicity index values or limits. Test pollutants that are being evaluated include cadmium, atrazine, and dieldrin. These investigations should provide the ability for real-time monitoring of the quality and safety of source water and watershed ecology.

BACKGROUND

The quality and security of treated drinking water and drinking water sources has come under closer scrutiny in recent years. Issues ranging from ecological, public health and national security are under consideration. Recent events in the United States and abroad have forced many utility and government planners to consider the possibility that infrastructures and water supplies and sources may be vulnerable to various types of physical, chemical and biological contamination. The President's Commission on Critical Infrastructure Protection noted in 1996, "there is a growing awareness that urban water systems are vulnerable to both manmade and natural, but unpredictable, threats and disasters such as earthquakes and terrorist attacks."

In general, contamination of public water supplies and source water occurs accidentally. Various chemical, biological and parasitical contamination events occur each year in the water supplies and there is concern that these contaminants could be resistant to chlorination or that the disinfectant oxidant in contact with the chemical contaminant could cause significant changes in the water quality. There are concerns that introduction of contaminants into a water supply or treatment delivery system could cause illness before the contaminant is detected or remedial action is taken.

Collecting and assessing data can be done in real-time. Digital video recording, signal analysis and computer advances have made it possible to monitor and evaluate scores of water quality parameters, chemical reactions and nuances of an organism's behavior as they are exposed to toxic substances within seconds of contact to a source water. Most of these technologies are in the preliminary evaluation stages of development. These investigations should provide insight into the quality and safety of source water and watershed ecology.

Biomonitors have been in use in parts of Europe since the 1970s to monitor the water quality of rivers. Few such systems are in place in the United States, where water quality is monitored mainly through chemical analysis that looks for contaminants. A weakness in the strict chemical approach is that it only identifies the toxins that are analyzed for in the source water. Also, the synergistic effect of compounds of different substances cannot be assessed by a chemical profile. Unlike analytical instruments, biomonitors respond to mixtures of toxic compounds without precalibration.

Based on recent events, various U.S. utilities have purchased or are considering implementing biomonitoring technologies to monitor source waters and infrastructure water supplies. Bio-monitoring has the potential to assess the relative toxicity and quality of water supplies and sources by monitoring multiple behavior patterns of single or multiple organisms. However, the technology needs further significant research to demonstrate reproducibility of results. Additionally, the EPA is concerned that a false sense of security may develop if the technologies are not completely developed and understood.

At the U.S. EPA Test and Evaluation (T&E) Facility in Cincinnati, Ohio, research is directed by the U.S. EPA's National Risk Management Research Laboratory. The T&E Facility is a RCRA-permitted research and development pilot plant in which new technologies for treating hazardous municipal and industrial wastes are evaluated. The facility was constructed in 1979 to allow usage of a broad spectrum of technological approaches for treating liquid and solid wastes. A broad range of research and development activities for evaluating chemistry of pollutant destruction and pollution control devices and waste treatment technologies are conducted at the Facility. A Biomonitoring Room, which is a room within the facility, was constructed in 2000 to house the bio-monitors for this research. Management and technical support at the facility is provided under contract by IT Corporation.

APPROACH

The new age of Biomonitors are a class of devices made possible by fast computers for monitoring water quality in real-time, on line, at any point in the source. EPA is currently evaluating several sensors including the following: an Algae Toximeter, Daphnia Toximeter, Clam Monitor, and a Fish Monitor.

In addition to real-time bio-monitoring of water at the source, remote access to the data generated by these monitors is made available through Internet connection. The Biomonitoring Room at the Cincinnati T&E Facility is currently connected by pcAnywhere™ to host connections. The manufacturer and two other desktop hosts have dial-in connection capability to observe the status and even control the operation of the monitors.

A future web-site is in store for the monitors so that observation from any computer is a possibility.

THE BIOMONITORS

The U.S. EPA is evaluating the following technologies:

a) Algae toximeter
b) Daphnia toximeter
c) Clam monitor
d) Fish monitor

The primary objective of this research is to evaluate the discriminatory ability of the biomonitoring analytical results for various classes and groups of contaminants and evaluate the reproducibility of test results. The EPA plans to evaluate different classes of compounds and contaminants and develop a data file of the response and sensitivity of the various biomonitors to a plethora of contaminants at a range of concentrations.

Algae Toximeter[1]

The algae toximeter (shown in Figure 1) uses algae from a standardized culture which is established within the instrument. This instrument compares the effects of toxins on a portion of algae to the effect on algae caused by a clean water source (eg. Carbon filtered tap water). In the case of significant deviations between the sample water and the reference water, an alarm is generated by the instrument to alert the operator. In addition to monitoring toxic substances, the monitor can determine different amounts of chlorophyll within different algae classes. It can differentiate and profile the families of Blue-green algae, Green algae, Diatoms and Cryptophytes in the sample water. This is possible because each class can be differentiated by their pigments and therefore has a "fingerprint" pattern for recognition.

Figure 1 – The Algae Toximeter at the T&E Facility

The principle of operation is based on the determination of the fluorescence spectrum and kinetics of the algae. This has a decided advantage over growth-inhibition-bench-test for algae that lasts up to 72 hours. The fluorescence measurement takes only 10 minutes and the instrument can operate continually. The measurements are based on the fluorescent characteristics of chlorophyll and other pigments in the algae. The color and brightness (intensity) of the excitation light is measured in each sample upon exposure to a light impulse.

For the algae, the light energy is a "food" source. If the algal cells are damaged by toxins in the sample water, the light energy will not be used by the cell and even without an additional background light there will be a higher fluorescence response to the light impulse.

The so-called Genty Parameter shown below represents the inhibition process and provides a measure for algae activity:

$$\text{Genty Parameter} = 100 \times \frac{f_m - f_o}{f_m} \, [\%]$$

where f_o= fluorescence measured without background light and f_m = fluorescence measured with background light.

The test mechanism of this monitor is very similar to a Biological Oxygen Demand (BOD) test, as the effect on fluorescence is a measure of electron transfer. The time required for the conventional 5-day bench test cannot compete with the 10-minute response time of this instrument.

Daphnia Toximeter[2]

The Daphnia Toximeter (shown in Figure 2) uses a video camera to directly monitor the activity of the test organism, the common freshwater water flea. Computer software analyzes the images of the daphnia while they are exposed to sample water. The activity of each organism is monitored continuously to determine if changes occur in any or all of several parameters. The Toxicity Index, a parameter calculated from individual behaviors, gives an overall status of the daphnia during testing. If changes occur suddenly or dramatically, the Toxicity Index will rate the change and will give an alarm when alarm conditions (as set by the operator) are met.

Figure 2 – The Daphnia Toximeter at the T&E Facility

The behavioral parameters under continual evaluation (and mathematical reduction by the Toxicity Index) by the instrument are: average velocity, speed class distribution, average distance of organisms to each other, the 'curviness' of swimming patterns as determined by two fractal dimension equations, average altitude and the recognition rate of Daphnia. The contribution of these individual parameters to the Toxicity Index can be weighted differently by the operator thereby adjusting the sensitivity of the instrument.

Clam Monitor[3]

The behavior of bivalves in response to toxicity in water is seemingly simple – they will close their shells. The measurement of gape, or opening of the shells, and the frequency of this opening is generally accepted as a measure of stress to the organisms. Gape behavior is measured using proximity sensors which detect a stainless steel proxy attached to the shell of each clam. By comparing the organism's baseline, or normal, gape behaviors to that of a test water, information regarding toxicity of a water can be determined.

As with all biomonitors, data reduction and evaluation is more complicated than the simple observation of the behavior. The clam monitor uses a remote monitoring unit to transmit collected data. When this unit is deployed in the field, it is powered by four batteries with 90 amp-hour rated capacity. A solar array recharges the batteries during daylight.

Fish Monitor

Certain aquatic animals generate measurable bioelectric signals. The measuring device for this monitor is known as bioelectric action potential (BAP). The BAP signals are emitted by the fish and propagate into the surrounding water. These signals can be recorded as rhythmic analog signals representative of specific movement activities.

The fish swim in the sample water in side-by-side compartments the size of paperback books. A computer linked to electronic sensors charts every gill pulse and body movement[4]. Fish 'coughs' are interruptions in the normal gill movements and are known indicators of stress to fish. The frequency of these "coughs" contribute to an alarm if a majority of the fish in the fish monitor are affected for a sufficiently long period of time.

EXPERIMENTAL SETUP AND ANALYTICAL SUPPORT

The biomonitoring instruments currently operate at the T&E Facility in batch mode. Individual batches of baseline water and test water are prepared in the necessary volume daily for each test. Each batch of test water is analyzed for water quality parameters: pH, Dissolved Oxygen (D.O.), conductivity, total chlorine, alkalinity and hardness. Test waters are also analyzed for the contaminants tested. Organic compounds are analyzed by Gas Chromatography (GC) and metals are analyzed by Atomic Absorption (AA). Analytical samples are taken from the original batch of test water and from the effluent of each instrument.

DATA EVALUATION

An acclimation period is required for the organisms in the source water before changes in response to exposure to toxins can be evaluated. The experimental plan developed evaluates the biomonitoring process in three stages: baseline period to establish control condition, exposure period in which the biomonitor is exposed to the test water spiked with the contaminant, and a recovery period to observe if the toxins have had any permanent effects on the organisms.

Algae Monitor

The herbicide Atrazine was tested with this instrument. Figure 3 shows the response of the algae to exposure to 40 ppm Atrazine in labline water. The tested concentration of 40 ppm for Atrazine was based on the solubility limit for this compound in water at 25^{o} C. A sharp increase in inhibition is seen immediately after exposure. The profile of algal organisms present in the test is also displayed. The next phase of testing will involve evaluations at lower atrazine concentrations to determine the algae monitor detection limit for atrazine in labline water.

The increase in inhibition is seen on the graph immediately after exposure to atrazine. The total duration of the test was 24 hours, comprising 9 hours of baseline data collection, 6 hours of data collection with exposure to atrazine-contaminated water, followed by 9 hours of data collection during the recovery period.

Daphnia Toximeter

Over 50 tests have been performed at the U.S. EPA T&E facility with the Daphnia Toximeter. Three source waters have been tested: a reconstituted hard water, a lake water and a carbon-filtered water with hardness added (labline water). The contaminants evaluated to date in these waters include cadmium, gasoline, and dieldrin.

Data was again evaluated in the three test stages. A baseline (acclimation) period of 12-24 hours was employed. Observations during the baseline period showed that the most common instrument parameters affecting toxicity were distance, speed, and altitude. Seasonal variation of natural water impacted the results. The water quality parameters associated with these source waters are as follows:

Test Water	DO (mg/L)	pH	Alkalinity (mg/L)	Hardness (mg/L as $CaCO_3$)
Reconstituted Hard Water	8-10	7.9-8.2	110-122	186-210
East Fork Lake	7-10	8-8.8	80-100	120-165
Labline Water	7.5-9	7.9-8.6	65-80	150-208

The exposure period was set at two hours to emulate the effects of a source water plume. Four-hour exposure periods were also examined. A recovery period of 6 to 24 hours concluded the tests to evaluate if the organisms could return to a steady state or a pre-exposure set of behaviors.

The software of this instrument allows the user to examine each parameter individually. This is very useful to help identify trends in response to a class of compounds or even a particular source water. The eight behavioral parameters that contribute to the Toxicity Index are shown in Figure 4. The 'partial' alarm that is given by the individual parameters are integrated to the overall index value.

Figure 5 depicts the instrument alarm response at levels of around 0.5 ppm cadmium (as $Cd(NO_3)_2$)in East Fork Lake water. Three concentration of cadmium were tested. These correspond to 20, 100 and 500 times the maximum concentration limit (MCL) of 0.005 mg/L for cadmium. An alarm was achieved 100% of the time with high concentrations of the contaminant (2.5 ppm). An alarm was not achieved at the lowest tested concentrations (0.1 ppm). The mid-range concentration (~0.5 ppm) achieved alarms in about 50% of the tests.

Figure 6 shows the response of the Daphnia Toximeter to a concentration of 5 ppm gasoline in the source water. As can be seen in the figure, the instrument provided a sharp alarm in response to exposure to gasoline.

Preliminary tests with dieldrin have shown alarms at concentrations of approximately 0.1 ppm in labline water. These tests are currently ongoing.

FUTURE GOALS

A clam monitor is deployed in the Little Miami River near Cincinnati, Ohio. The clam monitor is scheduled for testing at the T&E facility in May, 2002. A fish monitor will be evaluated later in 2002.

After experience is gained in culturing each new organism and baseline conditions are established, several additional compounds will be evaluated. These responses will be compiled into a database that can be used a reference. Compounds slated for immediate testing are: cyanide, MTBE, as well as continuing tests of atrazine and dieldrin.

REFERENCES

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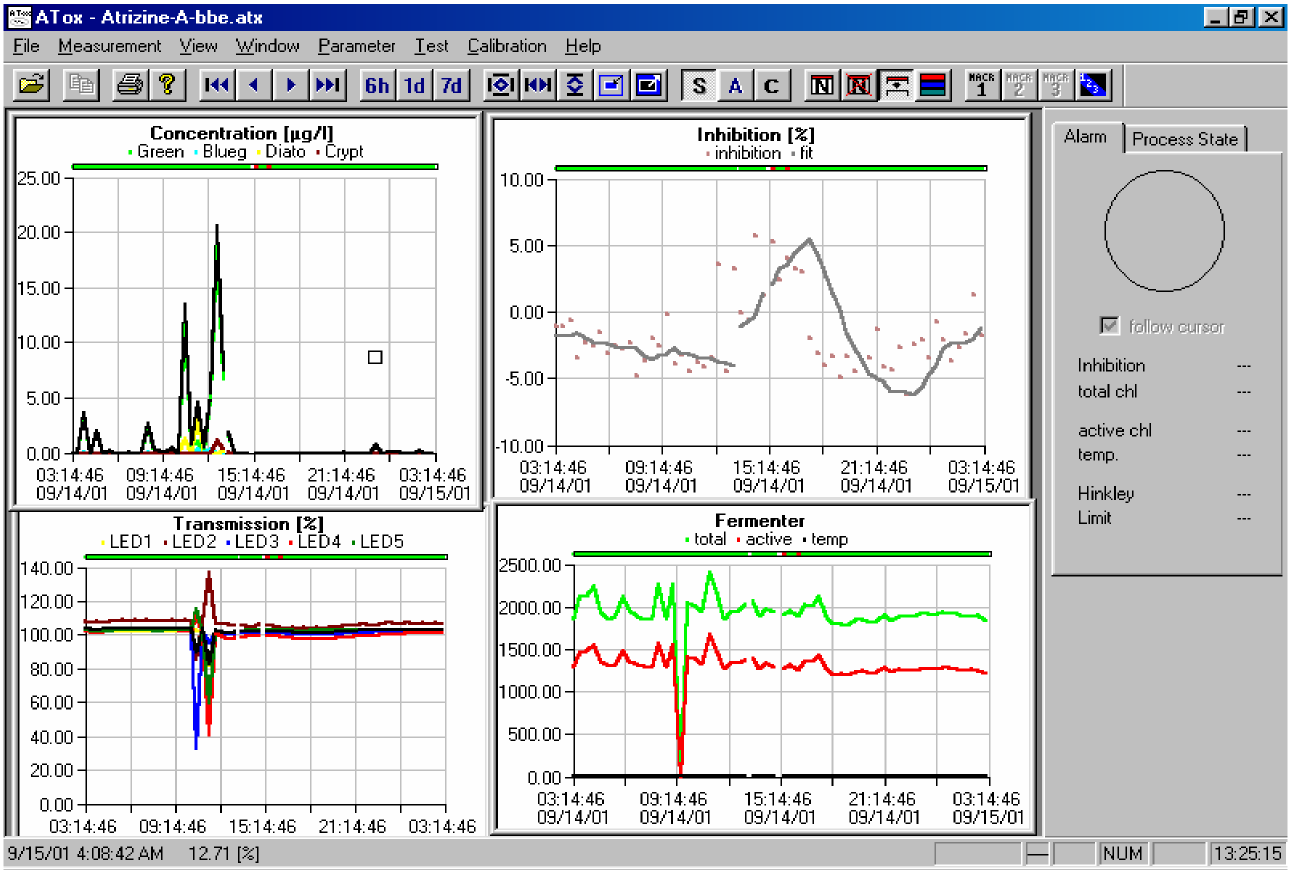

Figure 3. A snap shot of the screen of the Algae Toximeter during a 40 ppm Atrazine test.

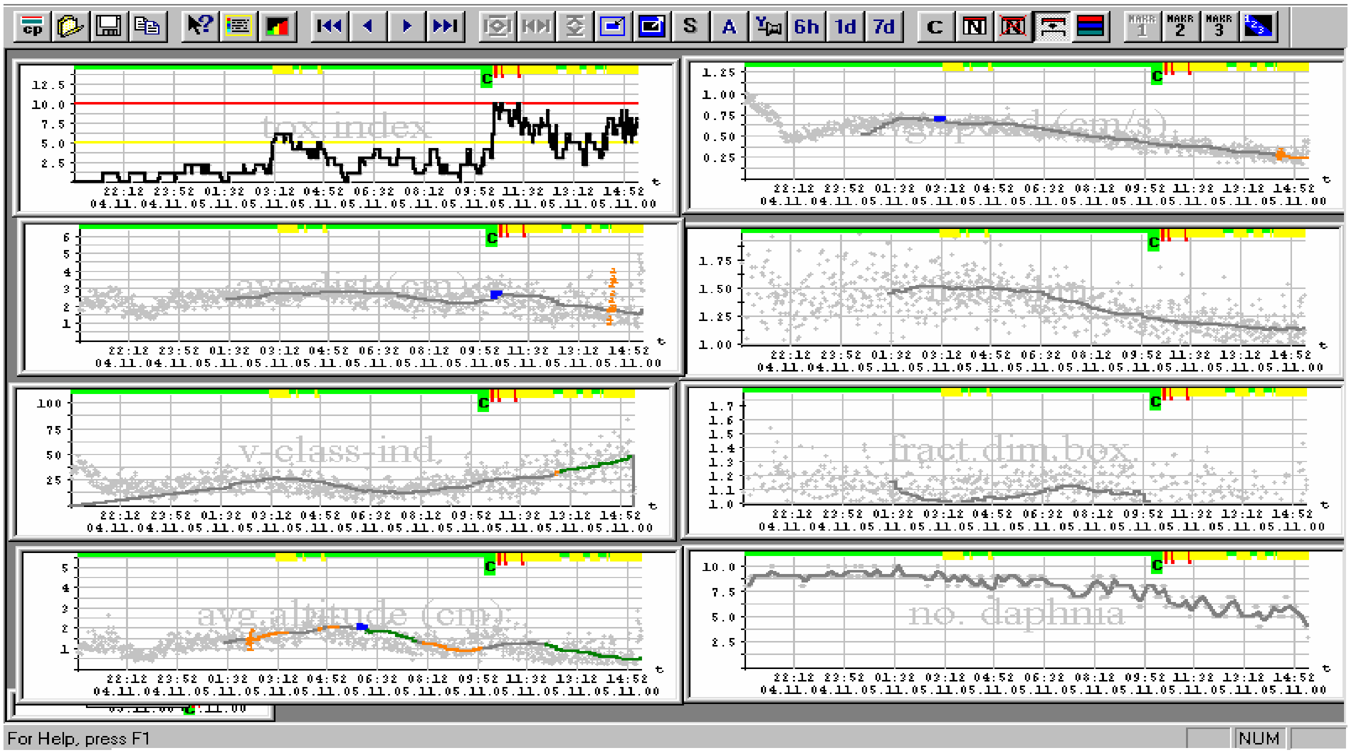

Figure 4 - Daphnia Toximeter test with all 8 parameters displayed.

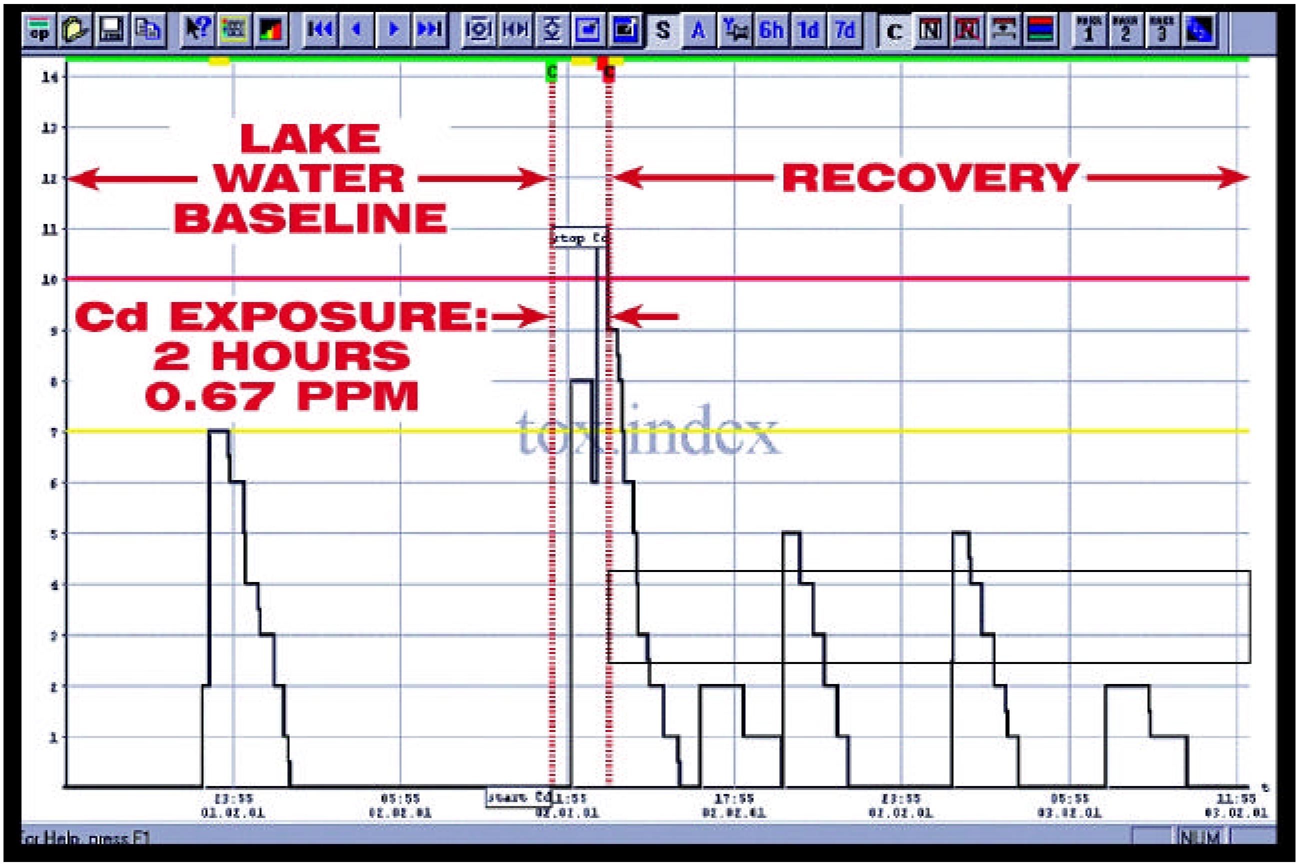

Figure 5. Daphnia Monitor Achieves Alarm in Response to Cadmium. The three test periods are indicated.

Figure 6. A high, sharp alarm is achieved by the Daphnia monitor in response to 5 ppm of Diesel Gasoline.